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Paul W. Kabler, et al

Robert A. Taft Sanitary Engineering Center
Cincinnati, Ohio

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VIRICIDAL EFFICIENCIES OF DISINFECTANTS IN WATER

Paul W. Kabler, M.D., Ph.D., F.A.P.H.A.; Norman A. Clarke, Ph.D.; Gerald Berg, Ph.D.; and Shih L. Chang, M.D., F.A.P.H.A.

Chief; in charge of Virus Disease Studies; Virologist; and in charge of Water Treatment Evaluations, respectively, of the Microbiology Section, Water Supply and Pollution Control Research

Robert A. Taft Sanitary Engineering Center
Public Health Service
U.S. Department of Health, Education, and Welfare
Cincinnati, Ohio

Recalling the recent outbreak of water-borne infectious hepatitis in New Delhi, India[1] and the discovery of more than 70 new human enteric viruses, it seems appropriate to review the information available regarding the viricidal efficiencies of disinfectants in water. The term "enteric viruses" as applied here includes the viruses which appear in large numbers in human feces and sewage, namely: Infectious hepatitis virus, Coxsackie groups A and B, poliovirus, adenovirus and the ECHO viruses. All types of these viruses are excreted in feces and can be found in urban sewage, especially in the late summer and early fall. Proved water-borne epidemics have occurred only with the virus of infectious hepatitis; however, outbreaks of poliomyelitis suspected of being water-borne have been reported.[2,3] Water-borne diseases caused by Coxsackie and ECHO viruses have not been recorded. Although no occurrence of adenovirus infection has been attributed to drinking water, three outbreaks of one of the clinical diseases caused by adenoviruses have been associated with swimming pools.[4,5,6]

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Chlorine

Because chlorine reacts with organic matter and forms combined chlorine compounds, reliable data on virus destruction depend on the chemical definition of the test water and determination of the types of titrable chlorine present, which are markedly influenced by the pH of the substrate. The rate of kill under fixed test conditions is proportional to the concentration of the kind of chlorine available. Sufficient demand-free virus material should be present to allow for accurate determination of the inactivation rate. Since many of the earlier studies on the destruction of viruses by chlorine did not meet these criteria, the resulting data are not included in this review. The reports before 1946 did not differentiate between free and combined chlorine, and many of the reports also showed such a high chlorine demand of the test system that the data on the viricidal efficiency of chlorine cannot be accurately interpreted.

Free chlorine

Information on the viricidal efficiency of free chlorine in water is presented in Table 1. The amounts of free chlorine recorded in this table for the studies of Chang,[7] Neefe[8] and Lensen[9-10] refer to the initial concentration. In each test the residual chlorine values were reduced to less than 1 mg/l. In the other investigations, the residual chlorine was not substantially less than the initial content.

The data of Weidenkopf[12] are probably most accurate because the test conditions are well defined and the plaque count technique provides an accurate estimation of virus concentration. Taking into consideration differences in procedures of the several investigators, the information in Table 1 reveals several significant points.

It is indicated that Theiler's virus is more resistant to the action of free chlorine than Coxsackie A2 virus, which is more resistant than poliovirus Type 1. Adenovirus Type 3[14] shows about the same resistance as do E. coli cells. The relative resistance of infectious hepatitis virus is not clear because sufficient contact times were not tested. It is apparent, however, that the different viruses exhibit wide variations in their susceptibility to free chlorine. The pH exerts marked effects on the viricidal efficiency of free chlorine. Weidenkopf's results show that lowering the pH from 7.0 to 6.0 reduces the required inactivation time by about 50 per cent, and the findings of both Weidenkopf and Clarke indicate that a rise in pH from 7.0 to 8.3-8.9 increases the inactivation period about six times.

In the destruction of viruses by chlorine, Clarke's work suggests that the temperature coefficient for a 10 degree change (Q_{10}) is in the range of 2 to 3, indicating that the inactivation time must be increased 2 to 3 times when the temperature is lowered 10°C. Data in Table 1 also indicate that the chlorine concentration coefficient (N) lies in the range of 0.7 to 0.9. This means that the inactivation time is reduced a little less than half when the free chlorine concentration is doubled. To increase virus kill, therefore, there is some advantage in increasing the contact time instead of raising the chlorine content.

Kelly and Sanderson[13] also observed that the viricidal efficiency of free chlorine is dependent on pH, temperature, chlorine concentration and exposure time. In addition, they pointed out that complete inactivation of some enteric viruses cannot be attained by exposure to 0.2 mg/l chlorine for 10 min. at pH 7.0 (usual bacterial parameters of disinfection) at an initial

level of 300 to 3000 virus doses per ml in the water.

In low chlorine residuals, the virus inactivation rate is markedly affected by temperature and pH. Data indicate that at temperature of approximately 20°C and pH values no higher than 8.0-8.5, a free chlorine residual of 0.2 to 0.3 mg/l will probably destroy most of the tested viruses in 30 minutes. At temperatures below 10 to 15°C and pH values greater than 8.5 effective virus kills with free chlorine residuals of 0.2 to 0.3 mg/l are probably not attainable unless long detention periods are used.

Combined chlorine

Trask et al.,[15] found that 5.4 and 8.1 mg/l of residual chlorine as chlorine-ammonia were required to destroy 90 per cent of Theiler's virus (FA strain) in 30 minutes, and in 10 minutes when the chlorine-ammonia weight ratio was 1 to 4. The temperature and pH of the treated water were not stated. At 26°C, a pH of 6.8 to 7.1, and a chlorine-ammonia weight ratio of 2 to 3, Chang et al.,[7] observed that 5.2 mg/l of residual chlorine killed only 85.7 per cent of Theiler's virus in 60 minutes. Trask and his associates also stated that 12.1 mg/l of titrable chlorine as azochloramid destroyed 90 per cent of Theiler's virus in 30 minutes, and Fair et al.,[16] found that 18 to 20 mg/l titrable chlorine as monochloramine T killed 60 to 80 per cent of Theiler's virus in 60 minutes at 27°C and pH of 7.0 to 7.2. These data clearly indicate the low viricidal efficiency of these compounds.

Kelly and Sanderson[17] showed that polioviruses (Type 1, MF 500 and Mahoney strains) and Coxsackie virus (Group B, type 5, EA 80) in water were inactivated by combined residual chlorine, the effective concentration

depending upon pH, contact time and strain of virus. In general, longer contact was required to destroy the Type 1 poliovirus than the Coxsackie B virus in this study, and increasing the pH decreased the rate of inactivation. At 25°C and a pH of 7, a concentration of at least 9 mg/l was necessary for inactivation of poliovirus with a contact period of 30 minutes, and of 6 mg/l with a one-hour contact period. Five-tenths mg/l required a contact period of more than seven hours. These data make it clear that present methods of disinfecting water with combined chlorine may not be adequate to destroy the more resistant virus strains.

Chlorine dioxide

Ridenour and Ingols[18] found chlorine dioxide to be as effective as free chlorine in inactivating poliovirus if the residuals are measured by the orthotolidine-arsenite (OTA) method. In 1953, Hettche and Schulz-Ehlbeck[19] reported that 0.08 mg/l chlorine dioxide, measured by the orthotolidine (OT) method, showed the same vircidal activity for poliovirus as 0.15 mg/l ozone or 0.25 mg/l free chlorine. However, Post and Moore[20] report that the OT and OTA methods make no distinction between chlorine and chlorine dioxide. Critical comparison of data on the viricidal efficiency of free chlorine and chlorine dioxide is, therefore, impossible if OT or OTA methods are used to measure the chlorine dioxide content.

Iodine

Recent reports[21-22] indicate that different enteric viruses may exhibit varying resistances to iodine and that Coxsackie A2 and B1 viruses

have about the same resistance to molecular iodine, but have twice the resistance of Type 1 poliovirus or ECHO 7 virus.

Berg et al.,[23] studied the destruction of several enteric viruses by elemental iodine (I_2) under carefully controlled conditions. Figure 1 shows the relationship of contact time and I_2 concentration required for 99 per cent destruction of each of five viruses tested at 15°C. The slopes of all of these lines are about the same and indicate that a change in contact time will have a slightly greater effect than a proportional change in the iodine concentration. Coxsackie A9 virus was the most resistant virus tested, requiring 10 mg/l of I_2 to destroy 99 per cent of the agent in 34 minutes at 15°C. At the same temperature and I_2 concentration, 99 per cent of ECHO 7 virus was killed in 3½ minutes. Type 1 poliovirus, Lotshaw strain, was almost as resistant as Coxsackie A9 virus, while the resistance of Type 1 poliovirus, Mahoney strain, and ECHO 9 virus fell in between the two extremes. With essential conditions constant, about 10 times longer contact time was required to destroy the most resistant virus as was needed to destroy the least resistant virus tested. In other comparative tests, at 25°C, 99 per cent of Coxsackie A9 virus was destroyed in 52 minutes by 1.0 mg/l of I_2, while the same concentration of I_2 (extrapolated data) destroyed 99 per cent of E. coli in 0.03 minutes, a difference of over 1700 times.

Iodide and iodate ions are completely inert as viricides. Triiodide is either inert or acts so slowly, that it is of little importance under most test conditions. Hypoiodous acid (HOI), which increases in concentration as the pH of the water increases, appears to be a more potent viricide than I_2.

Ozone

Kessel et al.,[24] reported that 0.05 to 0.45 mg/l ozone destroyed the same quantity of poliovirus in 2 minutes as did 0.5 to 1.0 mg/l residual gaseous chlorine in 1½ to 3 hours. Because a crude-virus suspension was used in the test and the types of residuals were not differentiated, it seems likely that most of the residual chlorine was in the combined form. The findings of Hettche and Schulz-Ehlbeck[19] also indicated that ozone was somewhat more vircidal for poliovirus than was free chlorine on a weight basis.

Ultraviolet light

The studies of Habel and Sockrider,[25] Murray et al.,[26] Milzer,[27] Oppenheimer[28] and others have shown that many viruses and vaccines including enteroviruses in liquid suspensions can be inactivated by ultraviolet irradiation. Carlson et al.,[29] found that ultraviolet irradiation was more effective in destroying poliovirus in water than coagulation and sedimentation, filtration, activated charcoal or storage, although their results cannot be considered a quantitative measure of the effectiveness of these methods. Coxsackie A and Theiler's virus in water were more resistant than E. coli to ultraviolet light in studies by Gilcreas and Kelly.[30] One major difficulty in ultraviolet light sterilization of water is the lack of a rapid field test to determine whether the water has been adequately treated. It is therefore necessary that the treatment unit be equipped with an internally situated ultraviolet energy source that supplies a stable energy application, and that this incident energy penetrate the fluid and provide

an essentially uniform density throughout the fluid as it passes through the effective radiation area.

Summary

The information and discussions presented appear to support the following summarizations:

1. Different enteric viruses exhibit varying resistances to free chlorine. Poliovirus, Coxsackie, and some ECHO viruses seem to be more resistant than coliform or enteric pathogenic bacteria. The free chlorine residuals required for inactivation are interdependent on pH, temperature and contact time.

2. Combined chlorine is considerably less viricidal than free chlorine, requiring higher concentrations and/or longer contact periods to achieve comparable inactivation.

3. Iodine is an effective viricide, but requires greater residuals and longer contact than hypochlorous acid.

4. Chlorine dioxide, ozone and ultraviolet light may be useful disinfectants, however, their comparative efficiency in water to free chlorine, and the quantitative effects of pH and temperature have not been established.

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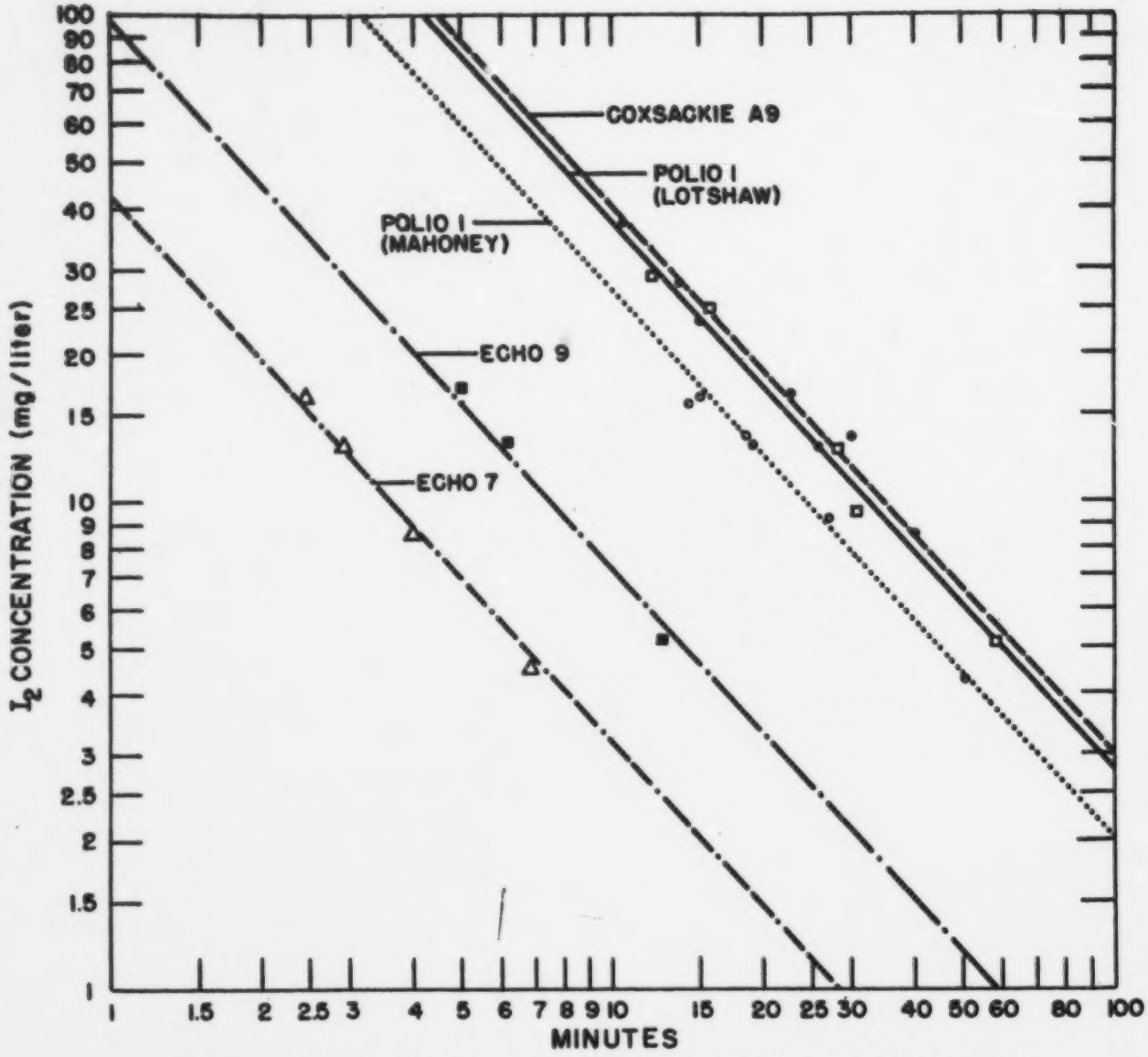
TABLE I. VIRICIDAL EFFICIENCY OF FREE CHLORINE IN WATER

Investigator	Virus	Temp. °C	Final pH	Free chlorine mg/l	Virus destruction
Chang et al. (7)	Part. purif. Theiler's virus in tap water	25 - 27	6.5 - 7.0	4.0 - 6.0	98.6% in 10 min.
		25 - 27	6.5 - 7.0	4.0 - 6.0	99 % in 5 min.
Neefe et al. (8)	Feces-borne Inf.Hepat. virus in dist. water	Room	6.7 - 6.8	3.25	30 min. cont. time protected all of 12 volunteers
Lensen et al.(9,10)	Purif. Polio II in dist. and lake water	19 - 25	7.4 - 7.9	1.0 - 1.5	10 min. cont. time protected all of 164 inoc. mice
Clarke and Kabler(11)	Purif. Coxsackie A2 in demand-free water	3 - 6	6.9 - 7.1	0.58 - 0.62	99.6% in 10 min.
		3 - 6	6.8 - 7.1	1.9 - 2.2	99.6% in 4 min.
		3 - 6	6.9 - 7.1	3.8 - 4.2	99.6% in 2½ min.
		3 - 6	8.8 - 9.0	1.9 - 2.0	99.6% in 24 min.
		3 - 6	8.8 - 9.0	3.7 - 4.3	99.6% in 9 min.
		3 - 6	8.8 - 9.0	7.4 - 8.3	99.6% in 5 min.
		27 - 29	6.9 - 7.1	0.16 - 0.18	99.6% in 4 min.
		27 - 29	6.9 - 7.1	0.44 - 0.58	99.6% in 3 min.
		27 - 29	8.8 - 9.0	0.10 - 0.18	99.6% in 10 min.
		27 - 29	8.8 - 9.0	0.27 - 0.32	99.6% in 7 min.
		27 - 29	8.8 - 9.0	0.92 - 1.0	99.6% in 3 min.
Weidenkopf (12)	Purif. Polio I(Mahoney) in demand-free water	0	6.0	0.39	99.6% in 3½ min.
		0	6.0	0.80	99.6% in 1½ min.
		0	7.0	0.23	99.6% in 8 min.
		0	7.0	0.53	99.6% in 4½ min.
		0	8.5	0.53	99.6% in 16 min.
		0	8.5	1.95	99.6% in 7½ min.
		0	8.5	5.00	99.6% in 3 min.
Kelly and Sanderson (13)	Purif. Polio I(Mahoney) in demand-free water	25 - 28	7.0	0.21 - 0.30	99.9% in 3 min.
		25 - 28	9.0	0.21 - 0.30	99.9% in 8 min.
	Purif. Polio III(Saukett) in demand-free water	25 - 28	7.0	0.11 - 0.20	99.9% in 2 min.
		25 - 28	9.0	0.11 - 0.20	99.9% in 16 min.
	Purif. Coxsackie B5 in demand-free water	25 - 28	7.0	0.21 - 0.30	99.9% in 1 min.
		25 - 28	9.0	0.21 - 0.30	99.9% in 8 min.
		1 - 5	7.0	0.21 - 0.30	99.9% in 16 min.
		1 - 5	8.0	0.21 - 0.30	99.9% in 30 min.
Clarke et al. (14)	Purif. Adenovirus 3 in demand-free water	25	8.8 - 9.0	0.20	99.8% in 40-50 sec.
		25	6.9 - 7.1	0.20	99.8% in 8-16 sec.
		4	8.8 - 9.0	0.20	99.8% in 80-100 sec.
		4	6.9 - 7.1	0.20	99.8% in 8-10 sec.



CONCENTRATION-TIME RELATIONSHIP FOR 99% DESTRUCTION OF ENTEROVIRUSES BY I_2 AT 15°C

FIGURE 1